LECTIN PROSPECTING IN COLOMBIAN LABIATAE. A SYSTEMATIC-ECOLOGICAL APPROACH – III. MAINLY EXOTIC SPECIES (CULTIVATED OR NATURALISED)

Prospección de lectinas en especies de Labiadas colombianas. Un enfoque sistemático-ecológico – III. Principalmente especies exóticas cultivadas o naturalizadas

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ABSTRACT

This is the third study of lectin and mucilage detection in Labiatae nutlets from Colombia. It was carried out on 30 taxa; 15 of them belonging to 14 genera in which no previous studies have been carried out in this field, the other 15 belonging to previously studied genera. A differential response was observed in the group of genera and species studied in terms of mucilage presence as well as lectin activity which consistently increased after extract treatment with Pectinex. Lectin activity was detected in 26 species, being important (more than 60% activity) in at least 75% of them. Genera such as *Aegiphila, Agastache, Ballota, Mentha* and *Origanum,* whilst not presenting mucilage, did present lectin activity, with high activity in most cases. This is the first time that a lectin has been reported in these genera. *Salvia* (in all but *Salvia* sections studied) presented mucilage and important lectin activity.

Key words. Labiatae, lectin, mucilage, *Agastache, Hyptis, Ocimum, Salvia, Stachys.*

RESUMEN

Este es el tercer estudio sobre detección de mucílagos y lectinas en núculas de Labiadas de Colombia. Fue llevado a cabo en 30 taxones, 15 de ellos pertenecientes a 14 géneros que no contaban con estudios previos en este campo y los otros 15 pertenecientes a géneros que ya contaban con alguna especie estudiada. Se observó una respuesta diferencial en el grupo de géneros y especies estudiados en lo que a presencia de mucílago y a actividad de lectina se refiere, actividad que se vió consistentemente incrementada con el tratamiento de los extractos con Pectinex. Se detectó actividad de lectina en 26 especies, y actividad importante (superior al

60% de actividad) en al menos el 75% de ellas. Los géneros *Aegiphila, Agastache, Ballota, Mentha* y *Origanum,* no presentaron mucílago y su actividad de lectina fue alta en la mayoría de los casos. Se registra actividad de lectina en estos géneros por primera vez. *Salvia* (en todas las secciones estudiadas a la fecha) presentó mucílago e importante actividad de lectina.

Palabras clave. Labiatae, lectina, mucilago, *Agastache, Hyptis, Ocimum, Salvia, Stachys.*

INTRODUCTION

The Labiatae family which according to current circumscription also groups several genera previously located in the Verbenaceae family (Cantino 1992a, 1992b, Wagstaff *et al*. 1998, Harley *et al*. 2004, Atkins 2004), is represented in Colombia by a total of 41 genera and *ca*. 308 species according to the latest work (Fernández-Alonso 2003, 2006, Pérez *et al.* 2006, Fernández-Alonso 2008a, 2008b). Colombia has 32 genera and 228 taxa (sp., subsp. and var.) from the traditionally recognized Labiatae, only a small part (17 spp.) consisting of foreign plants which have become naturalised to form an integral part of Colombian flora (Table 1), and also another 22 species from foreign Labiatae widely grown in Colombia for their various uses (Fernández-Alonso *et al*. 2003, Fernández-Alonso & Rivera 2006). The 9 new "Verbenaceae" genera, which are now dealt within the Labiatae, have a total of 80 native or naturalised species in Colombia (López-Palacios 1977, 1986, Rueda 1992, Aymard 2005, this work) and prospecting work in this particular area has just begun.

Continuing with prospecting of mucilages and lectins in the Labiatae family, focused on previous work carried out on Colombia native species (Fernández-Alonso *et al*. 2003, Pérez *et al*. 2006), the third contribution towards this ongoing work has resulted in cataloguing 24 non-native Colombian species belonging to 17 genera. As follows: a) 6 species currently naturalised in Colombia belonging to the *Leonurus* L.*, Ocimum* L.*, Salvia* L.*, Solenostemon* Tonn. and *Stachys* L., b) 13 species which are frequently cultivated in Colombia, belonging to *Agastache* Clayton ex Gronov.*, Hyssopus* L.*, Lavandula* L.*, Melissa* L.*, Mentha* L.*, Ocimum* L.*, Origanum* L.*, Rosmarinus* L.*, Salvia* L.*, Satureja* L. and *Thymus* L. and c) 5 exotic species (from the Iberian peninsula), non cultivated in Colombia, belonging to *Ballota* L.*, Sideritis* L. and sections *Aethiopis, Plethiosphace* of *Salvia* L. (Table 2 and Figures 1 and 2). Other 14 accessions of native species came from (belonging to the *Aegiphila* Jacq.*, Hyptis* Jacq.*, Ocimum, Salvia, Scutellaria* L. and *Stachys*) are also studied.

This combination let us extend our analysis of mucilages and lectins to a considerable number of species (112 accessions, belonging to 90 taxa) where these compounds have not been described before and also provided additional confirmatory evidence regarding genera studied in previous work (Fernández-Alonso *et al*. 2003, Pérez *et al*. 2006).

MATERIALS AND METHODS

Collecting and preserving botanical samples and fruits

The same procedures described by Fernández-Alonso *et al.* (2003) and Pérez *et al.* (2006) have generally been followed in that referring to collection itineraries and dates, herbarium sample-taking protocols, collecting nutlets and live material for culturing.

Table 1. Diversity and distribution of studied species.

Genus	Species in the world	Species in Colombia	Native studied species	Non Native studied species
Subfamily Lamioideae				
Ballota L.*	35	0	0	1 (e-ex)
Lamium L. *	40	2	0	0
Leonurus L. *	10	1	0	1 (e-nz)
Leonotis (Pers.) R. Br. *	15	2	0	1 (e-nz)
Marrubium L. *	30	1	0	0
Sideritis L.*	100	0	0	1 (e-ex)
Stachys L.	300	12	5	1 (e-nz)
Subfamily Nepetoideae				
Tribe Lavanduleae				
Lavandula L.*	30	0	0	1 (e-cv)
Tribe Mentheae				
Agastache Gronov. *	22	0	0	1 (e-cv)
Hyssopus L. *	5	0	0	1 (e-cv)
Lepechinia Willd.	30	10	6	0
Melissa L. *	3	1	0	1 (e-cv)
Mentha L. *	25	3	0	1 (e-cv)
Minthostachys (Benth.) Spach	12	3	1	0
Obtegomeria Doroszenko & P.D. Cantino	1	1	0	0
Origanum L.*	36	2	0	2 (e-cv)
Rosmarinus L.*	2	1	0	1 (e-cv)
Salvia L.	930	85	42	6 (e-nz, e-cv)
Satureja L.	180	13	0	1 (e-cv)
Thymus L.*	350	1	0	1 (e-cv)
Tribe Ocimeae				
Catoferia Benth.	4	1	0	0
Eriope Kunth ex Benth.	28	1	0	0
Hyptidendron Harley	16	1	0	0
Hyptis Jacq.	300	42	11	0
Marsypianthes Mart. ex Benth.	5	1	1	0
Ocimum L.	150	6	1	2 (e-cv, e-nz)
Plectranthus L´Her. *	200	3	0	0
Solenostemon Thonn*	10	1	0	1 (e-nz)
Tribe Prunelleae				
Prunella L.*	7	1	0	0
Subfamily Teucrioideae				
Aegiphila Jacq.	130	43	2	0
Ajuga L.*	40	1	0	0
Amasonia L.f.	8	4	0	0
Clerodendrum L.	450	8	0	0
Teucrium L.	100	1	0	0
Subfamily Scutellarioideae				
Scutellaria L..	300	22	3	0
Holmskioldia Retz. *	1	0	0	0
Subfam. Viticoideae				
Vitex L.	250	15	0	0
Gen. posición inct.				
Callicarpa L.	140	2	0	0
Cornutia L.	12	4	0	0
Gmelina L.*	35	2	0	0
Tectona L.*	3	1	0	0
Total 41	**4,345**	**298**	**72**	**24**

*: Non native genus; (e-ex.): exotic non cultivated or naturalized in Colombia; (e-nz): naturalised in Colombia; (e-cv): cultivated in Colombia.

Table 2. Diversity of species in the *Salvia* sections and number of exotic, colombian and endemic taxa studied.

Subgenus and sections of *Salvia*	Species in the world	Species in Colombia *	Endemic species in Colombia	Colombian * species analyzed	Exotic species analyzed
Subgen. *Calosphace*					
sect. *Angulatae* (Epling) Epling	60	20	16	8	0
sect. *xAngutes* Fern. Alonso	1	1	1	1	0
sect. *Carneae* (Epling) Epling	8	3	1	1	0
sect. *Flexuosae* (Epling) Epling	6	5	4	1	0
sect. *Hastatae* (Benth.) Epling	8	6	1	4	0
sect. *Killipiana* (Epling) Epling	5	5	5	1	0
sect. *Membranaceae* (Benth.) Epling	13	1	0	1	0
sect. *Potiles* Epling	1	1	0	1	0
sect. *Purpureae* (Epling) Epling	15	5	5	2	0
sect. *Rubescentes* (Epling) Epling	20	19	17	13	0
sect. *Secundae* Epling	10	1	0	1	0
sect. *Subrotundae* (Epling)Epling	3	1	0	1	0
sect. *Tubiflorae* (Epling) Epling	15	6	1	1	0
Other (8 sections -Colombia)	87	11	2	0	0
Subgen. *Salvia*					
sect. *Salvia*	200	1	0	1	2
Subgen. *Sclarea*					
sect. *Aethiopis* Benth.	6	0	0	0	1
sect. *Horminum* Benth.	10	0	0	0	1
sect. *Plethiosphace* Benth.	18	0	0	0	1
Total	486	86	53	37	5

* - Including native, naturalised or cultivated species in the country.

Itineraries. The plants included in this study came from different itineraries mainly carried out on the eastern cordillera of Colombia, where 14 accessions of native species came from (belonging to *Aegiphila* Jacq., *Hyptis* Jacq., *Ocimum, Salvia, Scutellaria* L. and *Stachys*) and 6 from naturalised exotic species, belonging to *Leonurus* L., *Leonotis* R. Br., *Ocimum, Salvia* and *Stachys* (Figures 1 and 2). The exotic species which are only found as cultivated plants in Colombia for their medicinal, culinary or ornamental uses represent another important group; 13 species belonging to *Agastache, Hysoppus, Lavandula, Melissa, Mentha, Ocimum, Origanum, Rosmarinus, Salvia, Satureja* and *Thymus* were analyzed. Five exotic wild species of *Ballota, Salvia* and *Sideritis* from central Iberian Peninsula fields of thyme (“tomillares”) were also studied (Appendix 1 and Figures 1 and 2).

Samples: The following specimens were collected in all cases:

a) Collecting fruit (nutlets) for erythroagglutination and enzyme-linked lectinosorbent assays (ELLSA) and mucilage;
b) Collecting herbarium control samples (about 180 collections) which were included as vouchers (Appendix 1) and nutlet samples for the project’s sample file (currently containing 150 accessions); and
c) Collecting live samples for culturing and follow-up in Bogotá’s Botanical Gardens, where around 50 taxa are being cultured. Control material, as well as fruit samples, were catalogued and deposited in the Colombian National Herbarium (COL) and many of these samples have been duplicated in the HUA, FMB, JBB, MEDEL herbaria, abbreviated according to Holmgren *et al*. (1990).

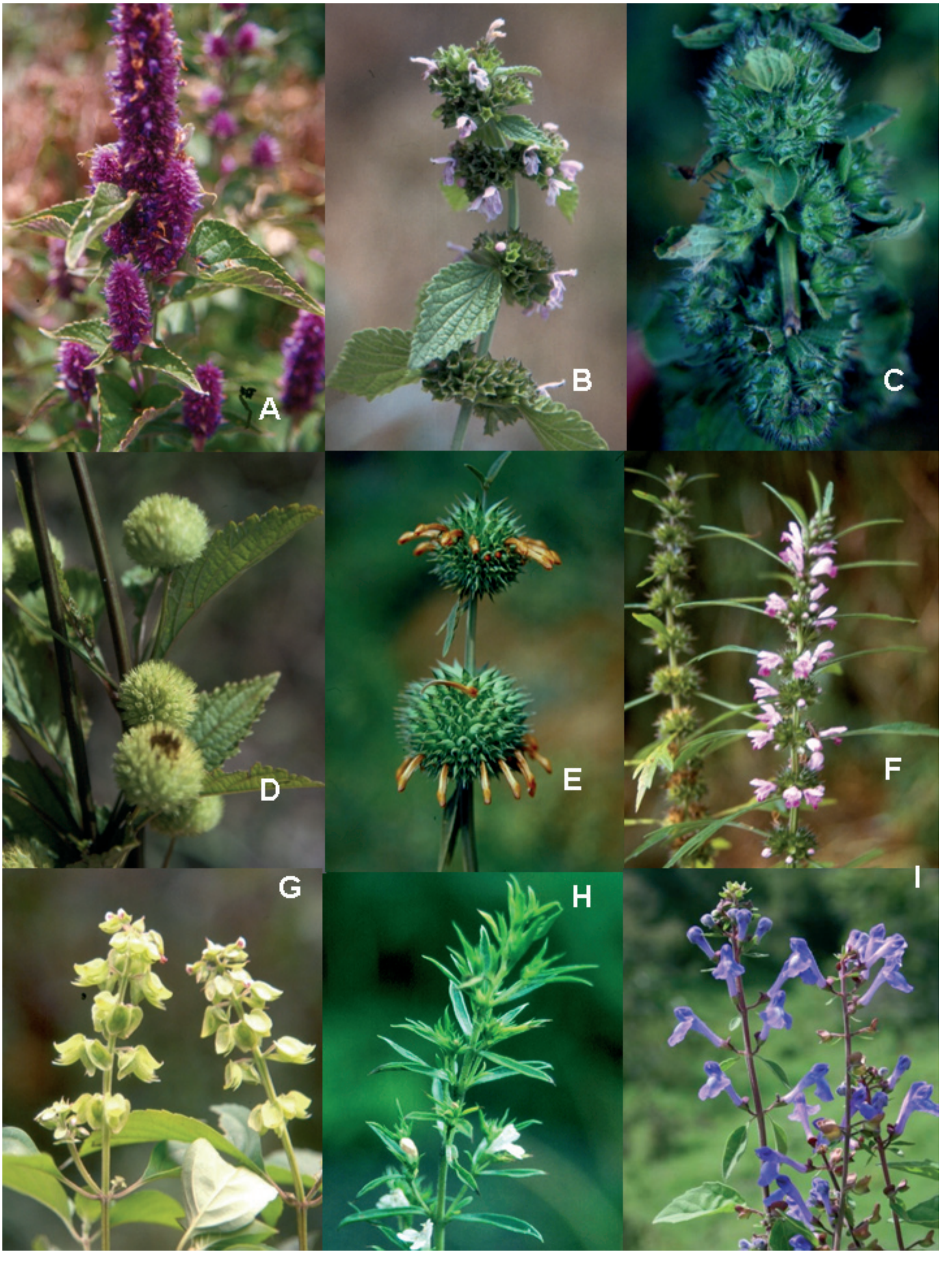

Figure 1. Some of the species studied . **A-** *Agastache rugosa* (Fisch. & C.A. Mey.) Ktze. **B-** *Ballota nigra* L. **C-** *Hyptis suaveolens* (L.) Poit. var. *mollisima* Fern. Alonso **D-** *Hyptis capitata* Jacq. **E-** *Leonotis nepetifolia* (L.) R. Br. **F-** *Leonurus japonicus* Houtt. **G-** *Ocimum campechianum* Mill. **H-** *Satureja montana* L. **I-** *Scutellaria purpurascens* Sw. subsp. *verecunda* (Epling.) Fern. Alonso (Photographs J.L. Fernández-Alonso, based on plants collected referred in the appendix).

Seeds produced by an hybrid originated in culture conditions (*S. x tunica-mariae* Fern. Alonso) were studied so that the results obtained with this plant could be compared to those from parental species.

A Labiatae nutlet reference collection has also been established with more than 480 accessions, some being stored in the nutlet/seed library in the Colombian National Herbarium and others in Bogotá's Botanical Garden's seed bank.

Types of growth and types of habitat in those taxa studied

All taxa studied have been catalogued according to habit type (growth type) and habitat (altitude at which taxa are found) to enable correlating these parameters with information resulting from mucilage and lectin assays. The biological/ecological function of mucilage is still not clear (even though various hypotheses have been suggested related to different aspects regarding germination) and no correlation has been established between the presence of mucilage and determined environmental conditions. Detailed classical types of habit (shrubs, subshrubs, scandent shrubs and perennial herbaceous and annual or biannual herbs) and habitat (tropical, subAndean, Andean and paramo) have been considered and described in previous work (Fernández-Alonso *et al*. 2003). The Tree biotype (Tr) has been added to the foregoing, being present in *Aegiphila cuatrecasana* Moldenke; regarding the types of habitat where samples came from, the following have been included for exotic species which are not present in Colombia: those having Mediterranean origin (E-Medit), subtropical origin (E-Subtr) and those having a Tropical origin (E-Tro), (Table 3).

Two variants having general characteristics (humid or dry) have been considered for each altitude range according to the amount of rainfall, delimiting dry areas as being those having a rainfall of less than 800-1,500 mm/year, depending on altitude. When a plant lives in more than one altitude range its least habitual altitude is given in parentheses.

Lectin extraction

Extraction was done with 20 mM phosphate buffer- 150 mM NaCl, pH 7.2-7.4 (PBS) in 1:10 (w/v) or 1:20 (w/v) ratio if a viscous solution appeared, seeds being left to soak in the solution for 2-3 h, at 4°C; they were then macerated and shaken at 4°C, for 16 h. The extract was spun at 39,000x*g* for 15 min at 4°C. The supernatant was used immediately or treated with Pectinex.

Pectinex treatment

Supernatant pH was adjusted to pH 4.7 with concentrated AcOH; 28 µl Pectinex Ultra SP-L (Novo) was then added per ml of extract. This was incubated at 28°C for 4 h, being occasionally shaken. The pH was adjusted to 7.0 with diluted NaOH and lectin activity was determined.

Lectin detection and quantification assays

Two assays were done to assess lectin presence:

a) Erythroagglutination. The assays were done on human RBCs as described (Pérez 1984) and on T/Tn-exposed RBCs. A+ human RBCs were enzymatically treated to expose T or Tn determinants (Hirohashi *et al*. 1985). Crude extracts were used in all cases. If appropriate, the haemoagglutination titre was determined and expressed as being the highest dilution where agglutination was still observed.
b) Lectin was also detected by ELLSA, according to the procedure described by Vega & Pérez (2006) for crude extracts treated with Pectinex and left un-treated. The plates were sensitized with asialo ovine submaxillary mucin (aOSM) isolate, using biotinylated *Vicia villosa* isolectin B4 as control (Tn antigen specific) and streptavidin peroxidase as detection system.

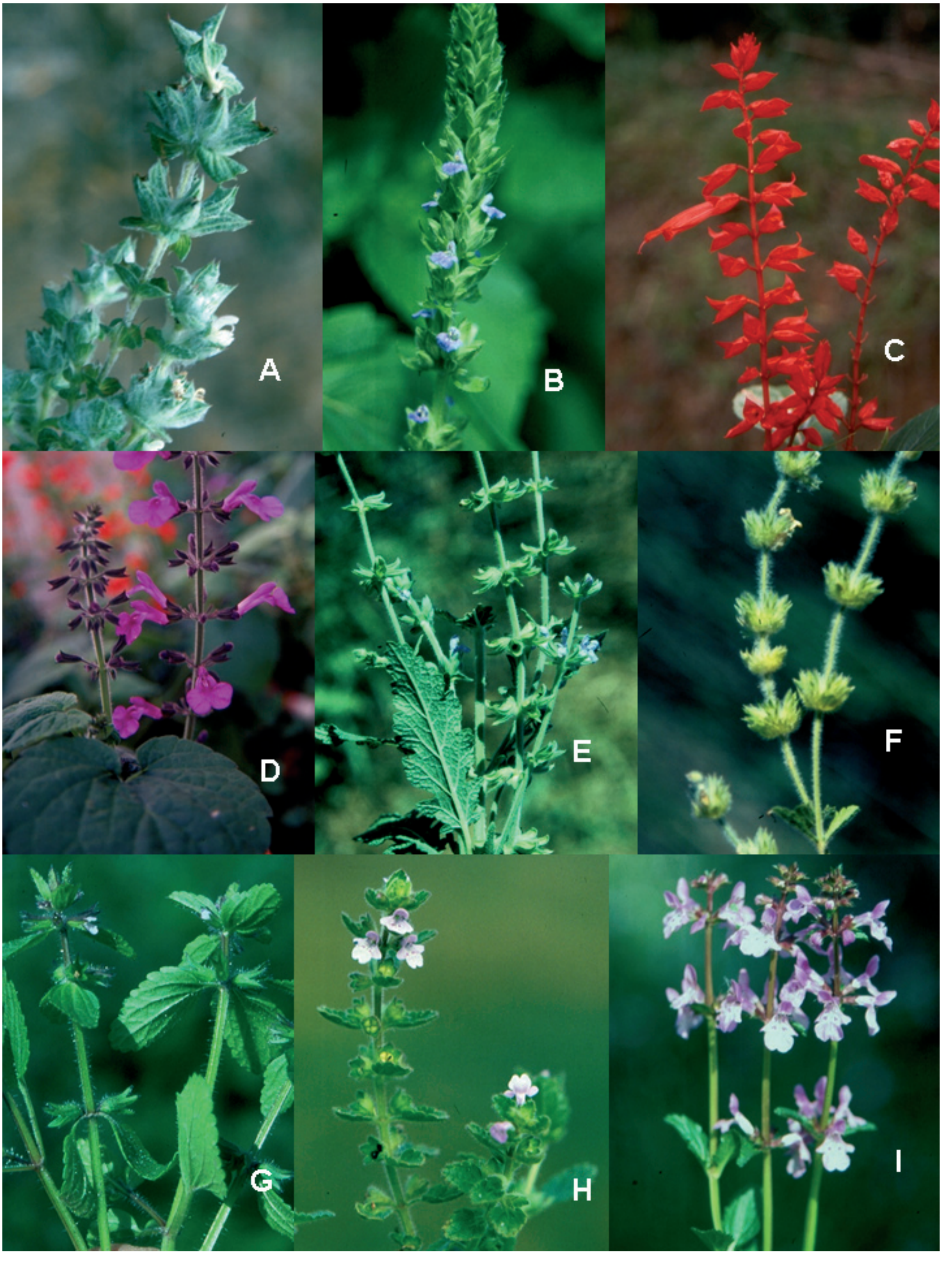

Figure 2. Some of the species studied. **A-** *Salvia aethiopis* L. **B-** *Salvia hispanica* L. **C-** *Salvia splendens* Sellow ex Schult. **D-** *Salvia x tunica-mariae* Fern. Alonso **E-** *Salvia verbenaca* L. **F-** *Sideritis hirsuta* L. **G-** *Stachys arvensis* L. **H-** *Stachys pusilla* (Wedd.) Briq. **I-** *Stachys radicans* Epling. (Photographs J.L. Fernández-Alonso, based on the plants collected and referred in the appendix).

Table 3. Lectin activity and presence of mucilage in Teucrioideae, Lamioideae, Scutellarioideae and in the Lavandulae and Ocimeae tribes from the Nepetoideae**.**

Species	Habit	Habitat	Muci-lage	Protein (mg/ml)	Lectin activity (%)	
					− Pectinex	+ Pectinex
Aegiphila cuatrecasana	Tr	And	No	0.20	38.2	32.6
Ballota nigra	P-her	E- Medit.	No	0.79	75.0	75.4
Hyptis capitata (Sta. Maria)*	P-herb.	Tro-h	No	1.25	0	9.1
H. pectinata (Santos)*	P-herb.	Tro-h	0.4	0.36	33.8	34.8
H. suaveolens var. *mollissima* (Santos)*	P-herb.	Sba-d	0.9	0.62	12.0	7.4
Lavandula vera	Sb-shr.	And/(E-Medit)	No	2.95	12.2	70.5
Leonurus japonica	A- her	Sba-h/ (Temp)	No	4.05	47.9	90.3
Leonotis nepetifolia	A- her	Sba-Tro/ (E-Subtr.)	No	0.23	4.1	8.4
Ocimum basilicum	Sb-shr	And-d	1.5	0.76	54.9	64.1
O. campechianum (Santos)*	A- her	Sba-Tro	0.8	0.19	16.4	8.8
O. gratissimum	P-her	Tro-d	0,2	1.16	3.3	45
Scutellaria purpurascens	P-her	Sba-Tro	No	2.36	3.8	6.3
Sideritis hirsuta	P-her	E-Medit.	No	7.55	71.3	72.8
Solenostemon scutellarioides	P-her	Tro/Sba-h	0.1	0.70	12.2	70.8
Stachys arvensis	A- her	(And)	No	1.92	64.1	96.4
S. pusilla	A- her	And	No	1.08	2.6	0

Habit. *A-her* : annual or biannual herbaceous ; *P-her*: perennial herbaceous ; *Shr: Shrub* 1-5 m; Sb-shr: Subshrub (having woody parts, being generally less than one meter in height); *Sc-shr*: Scandent shrub; *Tr:* tree. **Habitat**: *Tro:* Tropical area ranging from 0 – 1,000 m; *Sba:* Sub Andean area ranging from 1,000 – 2,200 m; *And*: Andean or High Andean area ranging from 2,200-3,300 m; *Par*: Paramo area ranging from 3,300 – 4,200 m; *E-Medit* (exotic Mediterranean), *E-Temp* (exotic from temperate zones); E-Subtr (exotic subtropical), *E-Tro* (exotic from tropical areas). *hu:* humid; *d***:** dry. When the place of origin is indicated in parenthesis (), this shows that the species has been cultivated in another region. * In the case of taxa previously studied from a different locality, the new locality is indicated. See also (Fernández-Alonso *et al*., 2003)

Mucilage assay

The mucilage assay was performed using fresh nutlets, following Hedge's recommendations (1970). A minimum 4-hour period was established for hydration (distilled water). Even though slight differences were detected in mucilage colour and degree of transparency, mucilage quantity was evaluated in terms of the following ratio: width of mucilage halo/seed width (smaller diameter), observed values varying between 0 and 3. Basic mucilage characteristics regarding colour, consistency and general appearance followed Hedge's terminology (1970).

RESULTS AND DISCUSSION

I- MUCILAGE PRESENCE

Macroscopic differences were observed amongst the taxa in which mucilage formation was presented in terms of their appearance, based on colour, consistency and degree of transparency; they could be grouped into four morphological types according to their characteristics which, in some ways, could be variations of the four basic ones (milky white-opaque, brown-opaque, translucent and transparent) recognized for species of *Salvia* by Hedge (1970). Thus, as this was not the object of the present work, information referring to the anatomical or micromorphological characterisation of types of mucilage in Labiatae has not been included, an aspect which has been treated for some taxa by authors such as Grubert (1974) or Martín-Mosquero *et al*. (2004). The four basic types are listed below, indicating in which species studied they have been observed. These morphological types are schematically represented (Figure 3), showing a possible relationship between them.

Type 1: - **Transparent and fibreless or having radiating cordons** (Figure 3-1), embraced by the mass of mucilage, having an egg-white aspect. This is only present in the four genera from tribe Mentheae (Nepetoideae): *Melissa, Rosmarinus, Salvia* (*S. amethystina* J.E. Sm. subsp. *amethystina* and *S. hispanica* L.) and *Thymus* and a *Hyptis* species (tribe Ocimeae): *H. pectinata* (L.) Poit.

Type 2: - **Transparent, irregularly fragmented and having conical processes** (detached from the nutlet) embraced by the mass of mucilage (Figure 3-2): Only observed in Nepetoideae, Ocimeae, in one species of *Ocimum* (*O. gratissimum* L.). This deals with a very distinctive type of nucula, due to the tuberculate ornament which it presents.

Type 3A: - **Milky white/pale, having flexuous fibres** in the mass of mucilage (Figure 3-3): Nepetoideae were present in both representatives of tribe Ocimeae: *Hyptis* (*H. suaveolens* (L.) Poit. var. *mollisima* Fern. Alonso) and Mentheae: *Salvia* (*S. coccinea* Ettling., *S. horminium* L. and *S. splendens* Sellow ex Roem. & Schult.). The cordons gave a "cottony" aspect to the mass of mucilage.

Type 3B: - **Transparent-Milky white/pale**. Mucilage having two concentric layers (a transparent external one and milky white/pale internal one) with flexuous fibres in a mass of mucilage (Figure 3-4). It represents just one variant of type 3. It has only been observed in *Salvia verbenaca* L., (Nepetoideae, Mentheae).

Type 4: - **Milky white/pale with fibres or radiating cordons** embraced by the mass of mucilage, forming a rigid mass in most cases (Figure 3-6). It is present in Mentheae (Nepetoideae): *Ocimum* (*O. basilicum* L.., *O. campechianum* Mill.) and *Salvia* (*S. aethiopis* L., *S. aratocensis* (Wood & Harley) Fern. Alonso, *S. sphaceliodes* Benth., *S. x tunica-mariae*). Cordons are less notable in *S. aratocensis* and *S. sphaceliodes* (Figure 3-5) and have a less consistent aspect.

There are ten genera within the group of taxa studied in which the formation of a mucilaginous mass due to moistening has not been observed (*Agastache, Ballota, Leonurus, Leonotis, Hyssopus, Lavandula, Mentha, Origanum, Satureja* and *Sideritis)*, partially agreeing with the consulted literature (Grubert 1974, Ryding 1992a, Budantsev & Lobova 1997, Ryding 2001, Martín-Mosquero *et al.* 2005). It was also presented in a species belonging to *Hyptis* (*H. capitata* Jacq.) and in two belonging to *Salvia* (*S. lavandulifolia* Vahl. and *S. officinalis* L.), which will be mentioned later on.

The Lamioideae, Scutellarioideae and Teucrioideae subfamilies

Concerning the species included within the subfamily Lamioideae, the results (Table 3) may be generalised as follows.

Traces (0.2) of mucilage were only observed in one species of *Stachys* (*S. micheliana* Briq. ex Mich.) of six studied, two in this work and four in previous work (Fernández-Alonso *et al*. 2003, Pérez *et al.* 2006). The absence of mucilage in this genus seems to be characteristic, bearing in mind that Ryding (1992a) obtained negative results in 7 species of *Stachys*. Other species from the Lamiodeae subfamily also did not present mucilage (Table 3), thereby corroborating and adding to Ryding's results (1992a) where myxocarpy was not detected in 4 species of *Ballota*, 2 from *Leonotis,* 2 from *Leonurus,* 7 from *Stachys* and 4 from *Sideritis*; the reports to date indicate that the absence of mucilage in nultles is generalised in this subfamily.

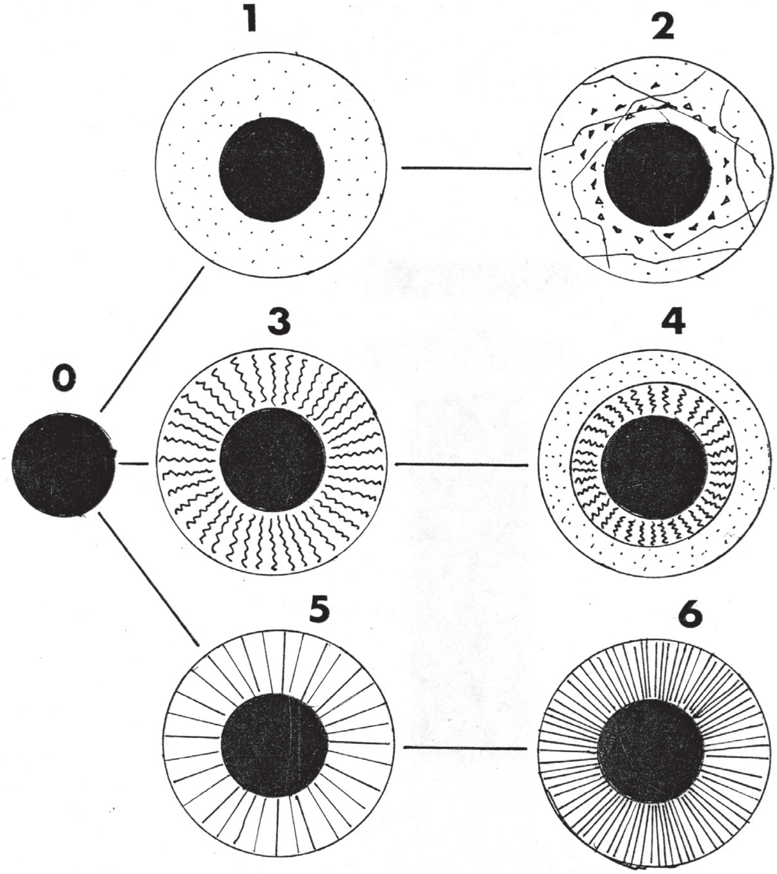

Figure 3. Schematic representation of the presence and types of mucilage in nultles (indicated with a black disc) of moistened Labiatae. 3**-0-** Nultles lacking mucilage. 3**-1.** Transparent and fibreless or radiating cordon mucilage (Type 1)**. 3-2.** Transparent, irregularly fragmented and embracing conical processes in the mucilage (Type 2). 3**-3.** Milky white/pale mucilage, with flexuous fibres (Type 3A). 3**-4.** Transparent-milky white/pale mucilage: mucilage having two concentric layers, the external one transparent and the internal having flexuous fibres (Type 3B). 3**-5.** Milky white/pale mucilage with fibres or radiating cordons, loosely set out/arranged (Type 4). 3**-6.** Milky white/pale and consistent with fibres or radiating cordons, densely set out/arranged (Type 4).

The absence of mucilage in nultles has been confirmed for *Aegiphila* (subfamily Teucrioideae), having two species studied (Pérez *et al*. 2006, this work), and *Scutellaria* (subfamily Scutellariodeae), having four species studied (Pérez *et al.* 2006, this work). Nevertheless, dealing with both cases of large genera (Harley *et al*. 2004), a greater number of species needs to be examined for having a more complete panorama concerning the occurrence of myxocarpy in them.

The Nepetoideae subfamily

On the contrary, most species analyzed in the Nepetoideae subfamily in this work presented myxocarpy, being extremely common in *Hyptis* and *Ocimum* (Table 4). These results agree with those described by Ryding (1992a) who found mucilage in 16 of 22 species of *Hyptis*, including *H. pectinata* and *H. suaveolens*. It is worth noting the absence of myxocarpy in *H. capitata*, corroborating Ryding report (1992a). The scarce amount of mucilage in *Ocimum gratissimum*, a species from Africa, is also worth noting (Table 4) when contrasting results obtained by ourselves in this work on other species of *Ocimum: O. basilicum* and *O. campechianum,* in previous work (Pérez *et al*. 2006) and in those described by Grubert (1974) and Ryding (1992b), who detected mucilage in 17 *Ocimum* taxa.

Within tribe **Mentheae** (subfam Nepetoideae), myxocarpy is habitual in genera such as *Salvia* and in the species analyzed from *Melissa, Rosmarinus* and *Thymus (M. officinalis* L.*, R. officinalis* L. and *T. vulgaris).* On the contrary, it is absent in species belonging to *Agastache, Hysoppus, Mentha, Origanum* and *Satureja* (Table 4). In the case of *Hyssopus officinalis* L., where we did not observe mucilage, there is contradictory evidence in the literature, as Ryding (1992a) did not observe mucilage whilst Grubert (1974) included this specie as having myxocarpy. The absence of mucilage in *Mentha spicata* indicates that there are species with and without mucilage within this genus, as Grubert (1974) found myxocarpy in seven species of *Mentha*, without analysing *M spicata* L.*,* whilst Ryding (1992a) cited (without specifying them) that some species of *Mentha* and *Origanum* are lacking mucilage.

The data compiled by Grubert (1974) revealed the presence of myxocarpy in 5 species of *Satureja* and in 20 species of *Thymus* whilst we did not observe mucilage in *Satureja montana* and a scant amount in *Thymus vulgaris* (0.5). It is worth specifying that the data of Grubert (1974) and Ryding (1992a, 1992b) are qualitative as, contrasting with this work, these authors did not evaluate the relative abundance of mucilage according to that proposed by Hedge (1970). It seems then that in the case of *Hyssopus*, *Mentha* and *Satureja* there may or may not appear mucilage, depending on the species. The results of myxocarpy in *Melissa officinalis* agreed with Grubert (1974) and those for *Rosmarinus officinalis* agreed with that found by Ryding (1992a) and Grubert (1974).

Of the Salvia species analyzed in this work only *S. lavandulifolia* and *S. officinalis*, belonging to *Salvia* subgenus and both having large seeds, did not present mucilage and in the rest of the species this was evident (0.7-3.0). It is worth pointing out that of the 40 species studied in this work and in our previous reports (Fernández-Alonso *et al.*, 2003, Pérez *et al.* 2006), 37 have mucilage without seeming to have a correlation with the type of habitat where they come from. Qualitative detection of myxocarpy in several species of *Salvia* studied here (*S. aethiopis, S. coccinea, S. hispanica, S. horminum, S. splendens* and *S. verbenaca*) is mentioned by Grubert (1974) and Oran (1997) in the case of *S. verbenaca*. We only found different results in the case of *S. officinalis,* a specie in which we did not observe mucilage presence. It should be stressed that no mucilage was also present in *S. lavandulifolia,* a specie which is taxonomically very close to *S. officinalis* (Figuerola *et al*. 1990), recently treated as being a subspecies of *S. officinalis* (Reales *et al.* 2004) and which had not any previous reports. The generalised occurrence of myxocarpy in the Salvia genus has been pointed out by Grubert (1974) who detected it in 90 species, by Hedge (1970) who only found three lacking mucilage after testing 40 Asian species, and by Oran (1997) who found it in 12 species which had not been previously examined by prior authors.

Table 4. Lectin activity and presence of mucilage in species from the Mentheae (Nepetoideae).

Species	Habit	Habitat	Muci-lage	Protein (mg/ml)	Lectin activity (%)	
					− Pectinex	+ Pectinex
Agastache rugosa	A- her	And/(Subtr)	No	0.83	22.3	37.4
Hyssopus officinalis	Sb-shr.	Medit./(And)	No	0.92	52.1	76.2
Melissa officinalis	P-her.	Medit./(And)	2.3	0.53	0	67.6
Mentha spicata	P-her.	Medit./(And)	No	0.83	49.7	77.9
Origanum vulgare	P-her.	Medit./(And)	No	1.28	0	74.7
O. mejorana	P-her/ Sb-shr.	Medit./(And)	No	1.38	48.3	52
Rosmarinus officinalis	Shr.	Medit./(And)	1.8	1.38	13.8	82.3
Satureja montana	P-her/ Sb-shr.	Medit./(And)	No	3.01	21.9	70.3
Thymus vulgaris	Sb-shr.	Medit./(And)	0.5	1.51	20.4	59.9
Salvia* Subgen. *Sclarea* Sect. *Aethiopis						
S. aethiopis	A-her	Medit	0.8	2.47	54.1	77.8
Subgen. *Sclarea* Sect. *Horminum*						
Salvia horminum	A-hert	Medit	1.8	2.67	69.6	86.9
Subgen. *Sclarea* Sect. *Plethiosphace*						
S. verbenaca	P-her.	Medit	0.85	0.79	7.6	45.1
Subgen. *Salvia* Sect. *Salvia*						
S. lavandulifolia	Sb-Shr	Medit	No	0.59	47.4	50.4
S. officinalis	Sb-Srb	Medit.	No	2.99	16	68
Subgen. *Calosphace* Sect *Angulatae*						
S. aratocensis	Sb-shr.	Sba-d	.8	0.31	22.5	11.0
subsp. *suratensis*	/Shr					
S. sphacelioides subsp. *pax-fluminensis* (cult)	Shr	And-h	0.5	2.62	11.3	10.1
S. sphacelioides subsp. *pax-fluminensis* (Soapaga)	Shr	Sba/And-d	0.7	0.36	6.8	11.5
Subgen. *Calosphace* Sect *x Angutes*						
S. x tunica-mariae	A-her/ P-her	Sba/And	1.0	0.1	2.8	10.0
Subgen. *Calosphace* Sect *Potiles*						
S. hispanica	A-her	And-d	2.3	0.84	16.3	79.7
Subgen. *Calosphace* Sect *Rubescentes*						
S. amethystina subsp. *amethystina*	P- her	And	1.8	0.13	3.4	14.2
Subgen. *Calosphace* Sect *Secundae*						
S. splendens (Tena)	P-her	Sba/And	1.5	2.1	87.0	87.5

II- LECTIN PRESENCE

IIA- ELLSA ASSAYS

Contrasting with that described in the literature for myxocarpy where a very considerable number of genera has been examined, the evidence concerning the presence of lectins in Labiatae until a few years ago was fundamentally limited to species native to species of *Salvia* natives of the Old World. The available information came from the reports of Bird & Wingham (1974, 1976, 1977, 1982) and in isolated contributions regarding *Hyptis suaveolens* (Bird 1960), *Salvia horminum* (Moore & Marsh 1975) and *Moluccella laevis* L. (Bird & Wingham 1969). A systematic study has recently been started regarding the presence of lectins in neotropical Labiatae (Fernández-Alonso *et al*. 2003, Pérez *et al*.

2006) where the presence of lectins has been detected in a considerable number of species belonging to different genera. The results presented below are part of this study.

A1- The Teucrioideae, Scutellarioideae and Lamioideae subfamilies (*Aegiphila, Scutellaria, Ballota, Leonurus, Leonotis, Sideritis* and *Stachys*)

Lectin directed against Tn antigen was detected for the first time in species belonging to *Ballota, Leonurus, Leonotis* and *Sideritis* (Table 4), considerably broadening the number of Labiatae where lectins are found. Due to the availability of material restricting analysis to just one species from each of these genera, other species must be assayed for discerning how generalised the presence of lectin is in them. Species where the presence of lectin have been established come from geographically very different localities and they are not taxonomically close within the subfamily (Iwarsson & Harvey 2003, Harley *et al.* 2004).

The experimental evidence indicates that ELLSA assay sensitivity and treating extracts with Pectinex facilitates the detection of these proteins. In the case of *Aegiphila cuatrecasana*, the level of lectin detected was similar with and without Pectinex treatment, compared to that observed with *Aegiphila bogotensis* (Spreng.) Moldenke (Pérez *et al.* 2006) where the effect of Pectinex treatment is evident, allowing greater lectin activity to be detected. It is worth stressing this result since it deals with taxonomically close species (Lopez-Palacios 1977, 1986). Something similar has been observed with five of the six species of *Stachys* studied (this work, Fernández Alonso *et al.* 2003, Pérez *et al.* 2006); however, lectin was never detected in the case of *S. pusilla* (Well.) Briq. The available data for *Scutellaria* (this work, Pérez *et al.* 2006) has shown great variability regarding the presence/absence of lectin, as two out of the four species did not present lectin. It would be recommendable to continue prospecting with other species from this genus given the presence of several taxa from this genus in Colombia (Fernández-Alonso, 2005).

A2- Tribes Lavandulae and Ocimeae from the Nepetoideae subfamily (*Lavandula, Hyptis, Ocimum* and *Solenostemon*)

Appreciable lectin activity was observed in *Ocimum* after digestion with Pectinex in two of the three species analyzed (Table 4), thereby confirming a previous report (Pérez *et al.* 2006) indicative of the presence of lectins able to recognise Tn antigen, this being corroborated by positive erythroagglutination assay results (Table 5).

The *Hyptis* species analyzed here had absence of lectin, even following treatment with Pectinex, except in *Hyptis pectinata* which showed activity (even though being relatively low). Similar lectin activity was observed (Fernández-Alonso *et al.* 2003) in a sample of *H. pectinata* from another locality, suggesting that there is a small amount of lectin in this specie. Nevertheless, the information about the presence of lectins in species belonging to this genus (Fernández-Alonso *et al.* 2003, Pérez *et al.* 2006), present very variable results regarding lectin activity, even within the same specie, as in the case of *H. capitata* samples coming from three different localities.

The other specie analyzed within the Ocimeae, *Solenostemon scutellariodes* (L.) Codd., an ornamental exotic species cultivated around the world, presented high lectin activity (70.8%), being the first report concerning the presence of lectins in this genus. This genus, which is widely diversified in tropical areas of the Old World (Suddée *et al.* 2004), seems to offer good prospects for prospecting for this type of protein.

Table 5. Erythroagglutinating activity in species of Labiatae.

Species	A +	B +	0 +	T	Tn
Aegiphila cuatrecasana	+4			+4	+4
Agastache rugosa	---			---	---
Ballota nigra	H	H	H	H	H
Lavandula vera	H	N.D.	N.D.	H	H
Hyptis capitata (Sta Maria)	---			---	---
H. pectinata (Santos)	+3			+4	+4
H. suaveolens var. *mollissima* (Santos)	+1			+2	+2
Leonurus japonica	H	H	H	H	---
Leonotis nepetifolia	---	---	N.D.	---	---
Mentha spicata	---	---	---	---	---
Ocimum basilicum	+1	---	---.	+1	+1
O. basilicum (cult)	---	---	---	+1	+2
O. campechianum (Santos)	+1			+2	+2
Origanum majorana	H	H	H	H	H
Rosmarinus officinalis	+3 (1:2)	N.D.	N.D.	+3 (1:4)	+4 (1:16)
Salvia aethiopis	+4 (1:8)[1]	+1	+2	+2	+3
S. hispanica	---	---	---	+4 (1:1)	+4 (1:11)
S. horminum	+4 (1:2)	N.D.	N.D.	+4 (1:4)	+4 (1:2)
S. verbenaca	+4 (1:16)	+3 (1:8)	+2 (1:4)	+3 (1:8)	+4 (1:16)
S. lavandulifolia	+1 (1:2)	+1	+1	+1	+1
S. officinalis	---	N.D.	N.D.	---	---
S. amethystina subsp. *amethystina*	+1			+4	+2
S. aratocensis subsp. *suratensis*	+3			+4	+4
S. x tunica-mariae	?			+4	+4
S, sphacelioides subsp. *pax-fluminensis* (cult)	?			+4	+2
S. sphacelioides subsp. *pax-fluminensis* (Soapaga)	?			---	---
Satureja montana	+4 (0)	N.D	N.D.	+4 (1:1)	+4 (1:1)
Scutellaria purpurascens	---	---	---	---	---
Sideritis hirsuta	H	---	---	H	H
Stachys arvensis	H	---	---	H	H
S. radicans	---	---	---	---	---
S. pusilla	---			---	---
Thymus vulgaris	---	---	---	---	---

Regarding tribe Lavandulae, lectin has been detected in *Lavandula vera* having considerable activity, this is the first report concerning the presence of lectin in this economically important genus having predominantly Mediterranean and subtropical distribution in the Old World (Upson 1997).

A3- Nepetoideae subfamily, tribe Mentheae (*Agastache, Hysoppus, Melissa, Mentha, Origanum, Rosmarinus, Salvia, Satureja* and *Thymus*.)

Within the species corresponding to the Nepetoideae subfamily, tribe Mentheae, lectin presence is very frequent, particularly in that referring to the *Salvia* genus (Table 4) where only four of the eleven species analyzed in this work did not show the presence of lectin. Similar results were obtained by Bird & Wingham (1974, 1976, 1977) who did not detect lectin in 12 out of 36 species analyzed. The presence of lectins in *S. aethiopis, S. horminum* and *S. verbenaca* detected by Bird & Wingham

(1974, 1976) was quantitatively confirmed by ELLSA assay (Table 5).

Similarly to that found in previous work (Fernández-Alonso *et al*. 2003, Pérez *et al.* 2006), the effect of Pectinex treatment in species from the Nepetoideae subfamily is notorious, thereby allowing the ELLSA assay to be used for detecting high lectin activity in species which otherwise would have been classified as lacking: it wich is the case of *Melissa officinalis, Origanum vulgare, Salvia amethystyna* subp *amethystyna,* S. x tunica-mariae and *S. sphaceloides* subsp. *pax-fluminensis*. Something similar happens in *S. hispanica, S officinalis* and *S. splendens* which had previously shown the absence of lectin with the erythroagglutination assay (Bird & Wingham 1974, 1976), in contrast to that observed by ourselves. A careful review of the pertinent literature showed that for all the taxa included in Table 4 this is the first time that the presence of lectin has been described in *Agastache, Hysoppus, Melissa, Mentha, Origanum, Rosmarinus, Satureja* and *Thymus.* On the other hand, information regarding *Salvia* has added 8 extra taxa, belonging to diverse infrageneric categories, some exclusive to the Old World. It is worth noting that cultivated species of Labiatae and thus having easily accessible nultles would not have been analyzed within the set of systematic investigations carried out during the 1970s by several groups in Europe or North-America (Bird & Wingham 1974, 1976, 1977, 1982, Moore & Marsh 1975).

IIB- ERYTHROAGGLUTINATION ASSAYS

The erythroagglutination results (Table 5) showed that all the *Salvia* species analyzed agglutinated Tn antigen-carrying erythrocytes, with the exception of *S. officinalis, S. melaleuca* Epling and *S. sphaceloides* sub *pax-fluminensis*. An important number of species recognized A- or T-determinants, frequently having lesser titres, meaning that one must be very careful when defining anti-Tn specificity; the presence of A1 antigen-specific lectin in *Hyptis suaveolens* var. *mollissima* Alonso described for the first time by Moore & Marsh (1975) is interesting in this context and this has been confirmed in the present work. Comparing these results with those obtained by ELLSA assay (Tables 3 and 4) it was observed that the presence of Tn antigen-recognising lectins could be detected in all species. However, the activity obtained with *S. officinalis* was not very high following Pectinex treatment, thereby explaining the negative erythroagglutination result since this is a less sensitive method.

A recent review of lectins present in the Labiatae family (Pérez & Vega 2007) synthesised the results obtained by other investigators regarding the presence of lectins in some of the species studied in this work. The available data indicates that it was not possible to detect the presence of these proteins through erythroagglutination assays on crude seed extracts of these species (Bird & Wingham 1974, 1976, 1977, 1982). This discrepancy is explainable if one considers that pectins interfering with detecting lectins were not eliminated in such work and that erythroagglutination is notoriously less sensitive than the ELLSA assay.

FINAL CONSIDERATIONS

The advantage of using a semi-quantitative method for evaluating myxocarpy (basically following Hedge's (1970) criteria) lies in the fact that it is relatively easy to use a stereoscope for discerning different morphological types of mucilage based on their external appearance, consistency and halo size. We have stressed (based on that described in this work) the absence of mucilage in *Salvia officinalis* and *S. lavandulifolia*, differently to that which occurs with most *Salvia* species genus.

Ryding's findings (1992a) were confirmed for the first species and such absence was also detected for the first time in the second, both forming a complex of very related Mediterranean taxa.

The results obtained in this and previous work (Fernández-Alonso *et al*. 2003, Pérez *et al*. 2006) have shown that lectin detection assay sensitivity becomes conspicuously increased if extracts have been previously treated with polygalacturonases (Pectinex) which digest the pectins (the main component of mucilage). This point is important as mucilage is abundant in many Labiatae species (particularly those belonging to the Nepetoideae subfamily). Using the ELLSA assay for detecting lectins instead of erythroagglutination offers greater sensitivity since it allows lower amounts of lectin to be observed at the same time as supplying semi-quantitative data about a protein's relative abundance. On the other hand, erythroagglutination using enzymatically treated erythrocytes (or untreated ones) leads to discriminating lectin specificity regarding T and Tn antigens and those antigens responsible for blood groups.

An additional question is raised regarding lectins' possible physiological role given that within a particular genus such as *Salvia,* where an important number of taxa have been analyzed (44), lectin is absent in around 10% of them. This could indicate that lectins are not an essential constituent in the seed or that they are replaced by protein having different specificity.

Considering the high number of existing Labiatae species and diversity and broad distribution of some of the larger genera, such as *Aegiphila, Clerodendrum, Hyptis, Ocimum, Plectranthus, Salvia, Scutellaria* and *Stachys* (Table 1), then it is evident that additional systematic prospecting work is required for having a broader panorama about the presence of mucilage and lectin in the nultles of different species. This will contribute towards understanding the functional and ecological significance of these compounds and also lead to extending the possibilities of using species from this family which have numerous types of economically important secondary metabolites, such as essential oils, antioxidant pigments, flavonoides and terpens.

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LITERATURE CITED

Atkins, S. 2004. Verbenaceae pp. 449-468, in: J.W. Kaldereit (ed.). Flowering Plants. Dicotyledons Lamiales (except Acanthaceae incluiding Avicenniaceae). *The Families and genera of Vascular Plants* VII. Spring Verlag, Berlin.

Aymard, G.A. 2005. Verbenaceae. pp. 407-445, in: P.E. Berry, K. Yatskievych & B.K. Holst (eds.) *Flora of the Venezuelan Guayana* 9. Rutaceae-Zygophyllaceae. Missouri Botanical Garden Press. St. Louis, Missouri.

Bird, G.W.G. 1960. Anti-A agglutinins from the seeds of *Hyptis suaveolens* Poit. Brit. J. Haematol 6: 151-153.

Bird, G.W.G. & J. Wingham. 1969. Anti-N from *Moluccella laevis*. Experientia 25: 1091.

Bird, G.W.G. & J. Wingham. 1974. Hemagglutinins from *Salvia*. Vox. Sang. 26: 163- 66.

Bird, G.W.G. & J. Wingham. 1976 More *Salvia* agglutinins. *Vox Sang.* 30: 217-219.

Bird, G.W.G. & J. Wingham. 1977. Yet more *Salvia* agglutinins. Vox. Sang. 32: 121-122.

Bird, G.W.G. & J. Wingham. 1982. More Tn-specific lectins from seeds of *Labiatae* genus: *Hyptis* sp. Chan, *Salvia lyrata* and *Marrubium velutinum*. Clin. Lab. Haematol. 4: 403-404.

Budantsev, A.L. & T.A. Lobova. 1997. Fruit morphology, anatomy and taxonomy of Nepeteae (Labiatae). Edinb. J. Bot. 54(2): 183-216.

Cantino, P.D. 1992a. Evidence for a polyphyletic origin of the Labiatae. Ann. Missouri Bot. Gard. 79: 361-379.

Cantino, P.D. 1992b. Toward a phylogenetic classification of the Labiatae. Pp 27-38, in: R.M. Harley & T. Reynolds (eds.). *Advances in Labiatae Science*. Royal Botanic Gardens. Kew.

Fernandez-Alonso, J.L. 2003. Estudios en Labiatae de Colombia IV. Novedades en *Salvia* y sinopsis de las Secciones *Angulatae* y *Purpureae*. Caldasia 25 (2): 235-281.

Fernández-Alonso, J.L. 2005. Estudios en Labiatae de Colombia V. Un nuevo nombre para *Scutellaria leptosiphon* Epling, planta redescubierta en la Cordillera Oriental de Colombia. Rev. Acad. Colomb. Ciencias 29 (112): 319-324.

Fernández-Alonso, J.L. 2006. Revisión taxonómica de *Salvia* Sect. *Siphonantha* (Labiatae). Anales Jard. Bot. Madrid 59 (2): 344-348.

Fernández-Alonso, J.L. 2008a. Estudios en Labiatae VI – *Salvia yukoyukparum*, nueva specie y primer representante de la Seccion *Tomentellae* en Colombia. Novon 18: 38-42.

Fernández-Alonso, J.L. 2008b. Estudios en Labiatae VII – Hibridación en el género *Salvia* en Colombia y su interés horticultural. Caldasia 30(1): 21-48.

Fernández-Alonso, J.L., N. Vega, J.J Filgueira, & G. Pérez. 2003. Lectin prospecting in Colombian Labiatae. A systematic-ecological approach. Biochem. System. Ecol. 31: 617-633.

Fernández-Alonso, J.L. & O. Rivera. 2006. Labiatae. pp. 385-582, in: G. Galeano & N. García (eds.): *Libro Rojo de Plantas de Colombia.* Volumen 3. Serie Libros Rojos de especies amenazadas de Colombia. Instituto Alexander von Humboldt – Instituto de Ciencias Naturales de la Universidad Nacional de Colombia – Ministerio de Ambiente, Vivienda y Desarrollo Territorial. Bogotá.

Figuerola, R., G. Stubing & J.B. Peris. 1990. El complejo taxonómico de *Salvia officinalis* (Sect. *Salvia*), en la región mediterránea occidental. Nomenclatura y nuevas combinaciones. Fol. Bot. Misc. 7: 81-86.

Grubert, M. 1974. Studies on the distribution of myxospermy among seeds and fruits of Angiospermae and its ecological importance. Acta Biol. Venez. 8: 315 - 551.

Harley R.M, S. Atkins, A.L. Budantsev, P.D. Cantino, B.J. Conn, R. Grayer, M.M. Harley, R. DE Kok, J. Krestoskaja, R. Morales, A.J. Paton, O. Ryding & T. Upson. 2004. Labiatae, pp. 167-275, in: J.W. Kaldereit (ed.). *Flowering Plants. Dicotyledons* Lamiales (except Acanthaceae including Avicenniaceae). The Families and genera of Vascular Plants VII. Springer Verlag, Berlin.

Hedge, I.C. 1970. Observations on the mucilage of *Salvia* fruits. Not. Royal. Bot. Gard. Edinburgh. 30: 79-95.

Hirohashi, S. H. Clausen, T. Yamada, T. Y. Shimosato & S-I. H. Hakomori. 1985. Blood group A cross-reacting epitope defined by monoclonal antibodies NCC Lu-35 and 81 expressed in cancer of blood group O or B individuals. Its identification

as Tn antigen. Proc. Natl. Acad. Sci. U.S.A. 82: 7039-7043.

Holmgren, P.K., N.H. Holmgren, & L.C. Barnett. (eds.) 1990. *Index Herbariorum*, I. New York Botanical Garden. New York.

Iwarsson, M. & Y. Harvey. 2003. Monograph of the genus *Leonotis* (Pers.) R. Br. (Lamiaceae). Kew Bull. 58: 597-465

Lopez-Palacios, S. 1977. *Flora de Venezuela* - Verbenaceae. Universidad de los Andes. Mérida.

Lopez-Palacios, S. 1986. Lista preliminar de las Verbenaceae existentes en Colombia con algunos de sus usos y nombres vulgares. Caldasia 15: 155-176.

Martín Mosquero, M.A., R. Juan & J. Pastor. 2004. Observaciones micromorfológicas y anatómicas en nuculas de *Prunella* L. y *Cleonia* L. (Lamiaceae) del suroeste de España. Acta Bot. Malacitana 29: 203-214.

Martín Mosquero, M.A., R. Juan & J. Pastor. 2005. Observaciones morfológicas y anatómicas en nuculas de *Ballota* (Lamiaceae) en el suroeste de España. Lazaroa 26: 35-40.

Moore, B.P.L. & S. Marsh. 1975. Identification of strong Sd(a^+) and Sda(a^{++}) red cells by hemagglutinins from *Salvia horminum*. Transfusion 15: 132-134.

Oran S.A. 1997. Nutlet anatomy of the genus *Salvia* in Jordan. Flora Medit. 7: 27-40.

Pérez, G. 1984. Isolation and characterization of a lectin from the seeds of *Erythrina edulis*. Phytochemistry 23 **:** 1229-1232.

pérez, G., N. Vega & J.L. Fernández-Alonso. 2006. Lectin prospecting in Colombian Labiatae. A systematic-ecological approach – II. *Caldasia* 28: 179-196.

Pérez, G. & N. Vega. 2007. Lamiaceae lectins. Functional Pl. Sci. Biotechnol. 1**:** 288-299.

Reales, A., D. Rivera, J.A. Palazón & C. Obón. 2004. Numerical taxonomy study of *Salvia* sect. *Salvia* (Labiatae). Bot. J. Linn. Soc. 145: 353-371.

Ryding, O 1992a. The distribution and evolution of myxocarpy in Lamiaceae. Pp. 85-96 in: R.M. Harley, T. Reynolds (eds.), *Advances in Labiatae Science*. Royal Botanic Gardens, Kew.

Ryding, O. 1992b. Pericarp structure and phylogeny within Lamiaceae subfamily Nepetoideae tribe Ocimeae. Nord. J. Bot. 12: 273-298.

Ryding, O. 2001. Myxocarpy in the Nepetoideae (Lamiaceae) with notes on myxodiaspory in general. Syst. Geogr. Pl. 71: 503-514.

Rueda, R.M. 1992. A new species of *Aegiphila* (Verbenaceae) from Colombia. Novon 2: 167-168.

Suddee, S., A.J. Paton & J.A.N. Parnell. 2004. A taxonomic revision of the tribe Ocimeae in continental South East Asia. I. Introduction, Hyptidinae / Hanceolinae. Kew Bull. 59: 337-378.

Upson, T.M. 1997. Systematics of the genus *Lavandula* (Lamiaceae). Ph. D. Thesis. Reading University.

Vega, N. & G. Pérez. 2006. Isolation and Characterisation of a *Salvia bogotensis* seed lectin specific for the Tn antigen. Phytochemistry 67: 347-355.

Wagstaff, S.J., L. Hickerson, R. Spangler, P.A. Reeves & R.G. Olmstead. 1998. Phylogeny in Labiatae s.l., inferred from cpDNA sequences. Pl. Syst. Evol. 209: 265-274.

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Appendix 1. Vouchers of species analyzed.

(e-ex.): exotic non cultivated or naturalized in Colombia; (e-nz): exotic naturalised in Colombia; (e-cv): exotic cultivated in Colombia.

Species	**Locality**	**Altitude (m)**	**Collector/ Herbarium**
Aegiphila *cuatrecasana* Moldenke	Col. Cundinam. (cult.)	2,600	JLF-24453 (COL)
Agastache *rugosa* (Fisch. & C.A. Mey.) Kuntze *(e-cv)*	Col. Cundinam. (cult.)	2,600	JLF- 23.923 (COL)
Ballota *nigra* L. *(e-ex)*	Spa. Valladolid: Encinas	750	JLF- 24152 (COL)
Hyptis *capitata* Jacq.	Col. Cundinam. S.Maria	800	JLF-23588 (COL)
H. pectinata (L.) Poit.	Col. Santander. Santos	1,400	JLF- 22040A(COL)
H. suaveolens (L.) Poit. var. *mollissima* Fern. Alonso	Col. Santander. Santos	1,400	JLF-22011 (COL)
Hyssopus *officinalis* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF-21386(COL)
Lavandula *vera* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF-21387(COL)
Leonurus *japonicus* Houtt. *(e-nz)*	Col. Santander, Tona	2,000	JLF- 21426(COL)
Leonotis *nepetifolia* (L.) R. Br.*(e-nz)*	Col. Santander. Santos	1,200	JLF-22010 (COL)
Melissa *officinalis* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF-21388(COL)
Mentha *spicata* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF-21389(COL)
Ocimum *basilicum* L. *(e-nz)*	Col. Santander, Suaita	1,800	JLF- 20868(COL)
O. basilicum L. *(e-cv)*	Col. Bogotá -mercado	2,600	JLF- 21869(COL)
O. campechianum Mill.	Col. Santander. Santos	1,400	JLF-22145(COL)
O. gratissimum L. *(e-cv)*	Col. Choco, Quibdó	100	JLF- 23918(COL)
Origanum *majorana* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF-21391(COL)
O. vulgare L. (e-cv)	Col. Cundinam. (cultiv)	2,600	JLF-22692(COL)
Rosmarinus *officinalis* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF-21392(COL)
Salvia *aethiopis* L. *(e-ex)*	Spa. Valladolid. Encinas	750	JLF- 24151 (COL)
S. amethystina J.E. Sm. subsp. *ametystina*	Col. Boyacá. Samacá	2,700	JLF-26260 (COL)
*S. aratocensis (*Wood & Harley) Fern. Alonso subsp. *suratensis* (Wood & Harley) Fern. Alonso	Col. Santander. Los Santos	1,500	JLF- 21918 (COL)
S. sphacelioides Benth. subsp. *pax-fluminensis* Fern. Alonso	Col. Boyacá. Soápaga	2,400	JLF- 23844 (COL)
S. sphacelioides Benth. subsp. *pax-fluminensis* Fern. Alonso *(e-cv)*	Col. Cundinam. (cultiv)	2,600	JLF-23772 (COL)
Salvia x *tunica-mariae* Fern. Alonso *(e-cv)*	Col. Cundinam. (cultiv)	2,600	JLF-23699 (COL)
S. hispanica L. *(e-nz)*	Col. Nariño. Tangua	2,200	JLF- 19953(COL)
S. horminum L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF- 21393(COL)
S. lavandulifolia Spreng. *(e-ex)*	Spa. Valladolid. Encinas	800	JLF- 24154 (COL)
S. officinalis L.(e-cv)	Col. Cundinam. (cultiv.)	2,600	JLF- 19807(COL)
S. splendens Sellow ex Schult.*(e-nz)*	Col. Cundinam. (cultiv.)	2,500	JLF- 21394(COL)
S. verbenaca L.*(e-ex)*	Spa. Valladolid. Encinas	750	JLF- 24153 (COL)
Satureja *montana* L. *(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF- 23395 (COL)
Scutellaria *purpurascens* Sw. subsp. *verecunda* (Epling.) Fern. Alonso	Col. Cundinamarca. S.Maria	800	JLF-23462 (COL)
Sideritis *hirsuta* L. *(e-ex)*	Spa. Valladolid. Encinas	850	JLF- 24155 (COL)
Solenestemon *scutellaroides* (L.) Codd. *(e-nz)*	Col. Cundinam. (cultiv.)	2,600	JLF- 21400 (COL)
Stachys *arvensis* L.*(e-nz)*	Col. Cundinam. Bogotá	2,600	JLF- 19521 (COL)
S. pusilla (Wedd.) Briq.	Col. Cundinamarca. Sopó	2,600	JLF- 23914 (COL)
S. radicans Epling	Col. Cundinamarca Sopó	2,600	JLF- 23266 (COL)
Thymus *vulgaris* L.*(e-cv)*	Col. Cundinam. (cultiv.)	2,600	JLF- 21401 (COL)